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Gradually Increasing Tidal Volume May Mitigate Experimental Lung Injury in Rats

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- High tidal volumes and pressures may worsen acute lung injury, sometimes resulting in a trade-off between inflicted damage *versus* inadequate ventilation

What This Article Tells Us That Is New

- In a rat model of experimental lung injury, gradually increasing tidal volume to a known injurious level may result in less (or in some circumstances, more) damage

ABSTRACT

Background: This study hypothesized that, in experimental mild acute respiratory distress syndrome, lung damage caused by high tidal volume (V_T) could be attenuated if V_T increased slowly enough to progressively reduce mechanical heterogeneity and to allow the epithelial and endothelial cells, as well as the extracellular matrix of the lung to adapt. For this purpose, different strategies of approaching maximal V_T were tested.

Methods: Sixty-four Wistar rats received *Escherichia coli* lipopolysaccharide intratracheally. After 24 h, animals were randomly assigned to receive mechanical ventilation with V_T = 6 ml/kg for 2 h (control); V_T = 6 ml/kg during hour 1 followed by an abrupt increase to V_T = 22 ml/kg during hour 2 (no adaptation time); V_T = 6 ml/kg during the first 30 min followed by a gradual V_T increase up to 22 ml/kg for 30 min, then constant V_T = 22 ml/kg during hour 2 (shorter adaptation time); and a more gradual V_T increase, from 6 to 22 ml/kg during hour 1 followed by V_T = 22 ml/kg during hour 2 (longer adaptation time). All animals were ventilated with positive end-expiratory pressure of 3 cm H_2O. Nonventilated animals were used for molecular biology analysis.

Results: At 2 h, diffuse alveolar damage score and heterogeneity index were greater in the longer adaptation time group than in the control and shorter adaptation time animals. Gene expression of interleukin-6 favored the shorter (median [interquartile range], 12.4 [9.1–17.8]) adaptation time compared with longer (76.7 [20.8 to 95.4]; P = 0.02) and no adaptation (65.5 [18.1 to 129.4]) time (P = 0.02) strategies. Amphiregulin, metalloproteinase-9, club cell secretory protein-16, and syndecan showed similar behavior.

Conclusions: In experimental mild acute respiratory distress syndrome, lung damage in the shorter adaptation time group compared with the no adaptation time group was attenuated in a time-dependent fashion by preemptive adaptation of the alveolar epithelial cells and extracellular matrix. Extending the adaptation period increased cumulative power and did not prevent lung damage, because it may have exposed animals to injurious strain earlier and for a longer time, thereby negating any adaptive benefit.

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In patients with acute respiratory distress syndrome (ARDS), mechanical ventilation with high tidal volume (V_T) promotes ventilator-induced lung injury and has been recognized as a major risk factor for organ failure and death.[1,2] High V_T as well as static (positive end-expiratory pressure [PEEP], respiratory system plateau pressure, driving pressure ($\Delta P_{,RS}$])[3] and dynamic parameters (respiratory rate [RR],[4] inspiratory[5] and expiratory[6] peak flow) have been implicated in ventilator-induced lung injury causation. However, most investigations of ventilator-induced lung injury have focused on the static characteristics of the individual tidal cycle,[7] whereas the dynamic characteristics and consequences of repetition intensity have been somewhat overlooked.[8] The knowledge that both dynamic and static respiratory variables may be injurious has led to the concept of damaging mechanical power transferred from the ventilator to the lungs.[9,10] Along this line, the absolute value of power itself is not the defining characteristic associated with ventilator-induced lung injury; its hazard is conditioned by the maximum tidal strain associated with that power level.[11–13]

This article is accompanied by an editorial on p. 680. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). N.S.F. and C.S.S. contributed equally to this article.

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For the individual patient, the stress and strain thresholds required to induce ventilator-induced lung injury are still unknown. They likely depend on the components and organization of the extracellular matrix, as well as on the preexisting degrees of epithelial and endothelial cell injury.[14] In acutely injured lungs, stepwise increases in V_T begin to cause damage when strain exceeds a critical level; injury is intensified with repetition of excessive stress.[11,15] In heterogeneous lungs with varying regional aeration, tidal energy can concentrate on a small mass of pulmonary tissue, producing, aggravating, or propagating lung injury with successive cycles. Nevertheless, when tidal strain is higher than normal but kept below this critical damage threshold, injury may be prevented, delayed,[16] or even quickly repaired.[17] In the clinical setting and in virtually all laboratory experiments to date, changes in V_T are usually applied abruptly. However, both normal and preinjured lungs behave as a viscoelastic system[18] in which elements of the extracellular matrix require an adaptive "stress relaxation" time to mitigate the damaging strain associated with large tidal excursions. These internal adjustments occur over both short and extended time scales,[18] depending on the degree of lung injury.

Based on the foregoing, we hypothesized that lung damage caused by an abrupt increase in V_T would be attenuated if V_T were increased slowly enough to reduce alveolar mechanical heterogeneity and to allow the epithelium, endothelium, and extracellular matrix to adapt to the ultimate high tidal volume level. To our knowledge, this intriguing possibility has not been investigated previously, and the time course of any adaptive potential has not been determined. Thus, the present study aimed to compare the nature and severity of lung damage incurred by different strategies of V_T increase: abrupt (no adaptation time), gradual (shorter adaptation time), and even more gradual (longer adaptation time). For this purpose, lung function, heterogeneity index, diffuse alveolar damage score, and expression of genes related to inflammation, alveolar stretch, epithelial and endothelial cell injuries, and extracellular matrix damage were evaluated in a rodent model of mild ARDS.

Materials and Methods

This study was approved by the Ethics Committee of the Health Sciences Center, Federal University of Rio de Janeiro (Comissão de Ética no Uso de Animais 086/17). The present study followed the ARRIVE guidelines for reporting of animal research.[19] Detailed methods are described in Supplemental Digital Content 1 (http://links.lww.com/ALN/B863).

Animal Preparation and Experimental Protocol

Sixty-four male Wistar rats (weight 376 ± 52 g) received *Escherichia coli* lipopolysaccharide (200 μg) intratracheally to induce ARDS.[20] At 24 h, 32 animals were anesthetized, paralyzed, and mechanically ventilated (V500; Dräger Medical, Germany) in volume-controlled mode with V_T = 6 ml/kg, PEEP = 3 cm H_2O, RR adjusted to $Paco_2$ = 35 to 45 mmHg, and fraction of inspired oxygen = 0.4 (fig. 1). They were then randomly assigned to receive one of four ventilation strategies (n = 8/group): mechanical ventilation with protective strategy (V_T = 6 ml/kg) for 2 h (control); V_T = 6 ml/kg during hour 1 followed by constant V_T = 22 ml/kg (high tidal volume) until a 2-h period was complete (no adaptation time); V_T = 6 ml/kg during the first 30 min followed by a gradual slow V_T (0.5 ml · kg^{-1} · min^{-1}) increase up to 22 ml/kg for 30 min, then constant V_T = 22 ml/kg during hour 2 (shorter adaptation time); and a more gradual V_T increase from 6 to 22 ml/kg (0.25 ml · kg^{-1} · min^{-1}) during hour 1 followed by constant V_T = 22 ml/kg during hour 2 (longer adaptation time). The V_T size of 22 ml/kg was chosen because it has been shown to promote ventilator-induced lung injury and yet allow survival for 2 h in this rodent model.[21] The gradual increases in V_T were done manually (fig. 1). Eight nonventilated animals served for comparison purposes in our molecular biology analyses (n = 8). Arterial blood gases and respiratory mechanics were measured. At the experiment's end, the animals were euthanized, and their lungs were extracted for histologic and molecular biology analysis.

To dissociate the effects of gradually increasing V_T from those of longer exposure to higher tidal volumes, we also evaluated the effects of these mechanical ventilation strategies at 1 h. For this purpose, 24 animals were ventilated in control, shorter adaptation time, and longer adaptation time groups (n = 8/group) and euthanized at 1 h. Heterogeneity index, diffuse alveolar damage score, and gene expression of biomarkers were then measured.

Echocardiography

Left ventricular ejection fraction, cardiac output, heart rate, and the ratio between pulmonary acceleration time and pulmonary ejection time (an indirect index of pulmonary arterial hypertension) were measured.

Data Acquisition and Processing

Airflow, airway (P_{aw}), esophageal (P_{es}), and transpulmonary (P_L) pressures were measured[22] every 30 min. Respiratory system (ΔP_{RS}) and transpulmonary (ΔP_L) driving pressures were calculated. Cumulative power exposure[12,13] was calculated at 1 and 2 h.

Histology

Slices (4 μm thick) were cut and stained with hematoxylin and eosin. The diffuse alveolar damage score was quantified[23] as was the heterogeneity index (β).[24,25]

Biologic Markers

The mRNA expression of interleukin-6, amphiregulin,[26] club cell secretory protein-16, vascular cell adhesion

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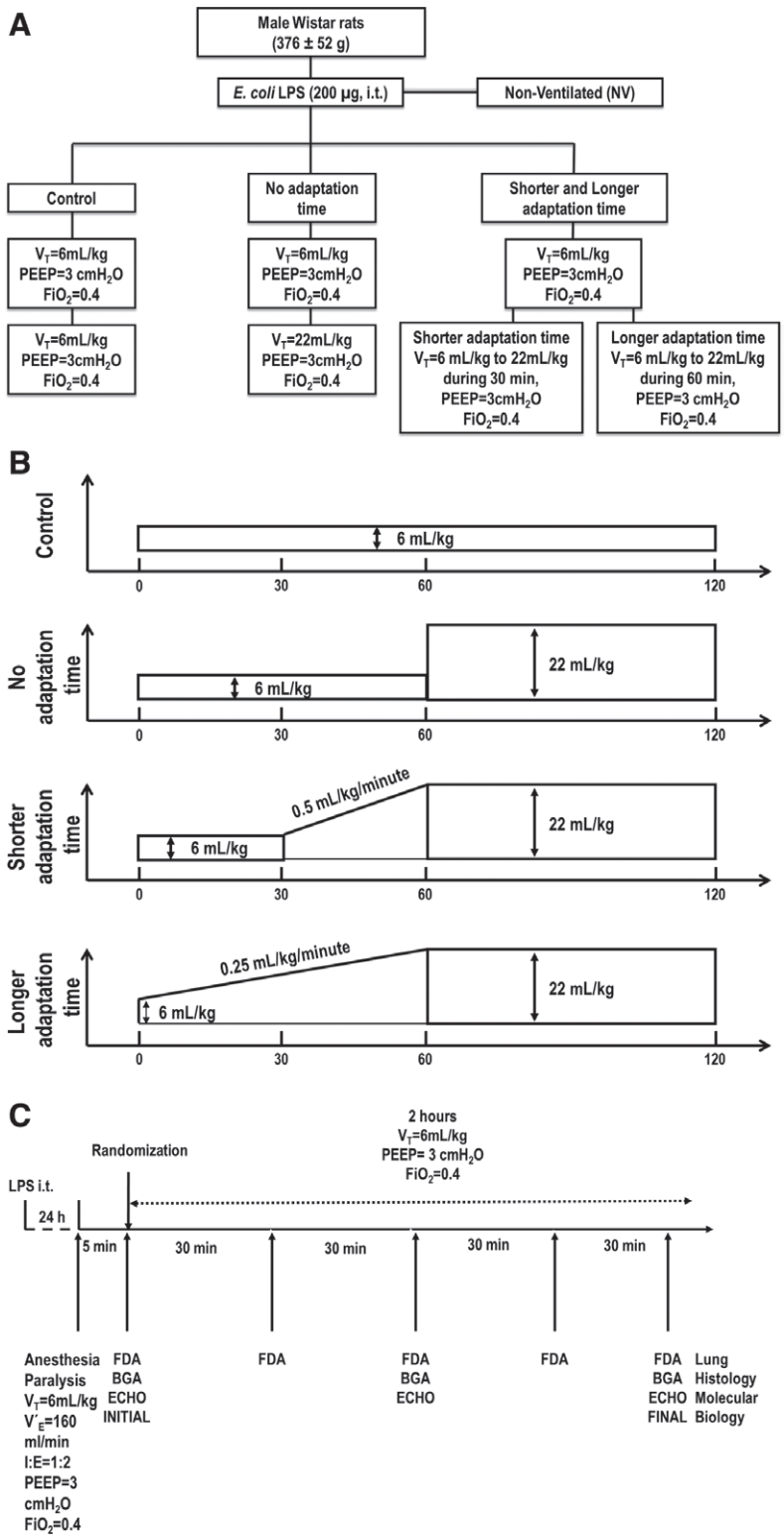

Fig. 1. Schematic flowchart of study design (*A*), protocol (*B*), and timeline representation of the experimental protocol (*C*). Lung mechanics were assessed every 30 min. Arterial blood gases were evaluated at the initial time, at 1 h, and at the final time. BGA, blood gas analysis; ECHO, echocardiography; FDA, functional data acquisition; FiO_2, fraction of inspired oxygen; I:E, inspiratory-to-expiratory ratio; i.t., intratracheally; LPS, *Escherichia coli* lipopolysaccharide; MV, minute ventilation; NV, nonventilated; PEEP, positive end-expiratory pressure; RR, respiratory rate; V_T, tidal volume.

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molecule-1, syndecan-1[27], metalloproteinase-9,[28] and decorin[29] in lung tissue was measured by reverse transcriptase-polymerase chain reactions. Primer sequences are listed in table 1 in Supplemental Digital Content 2 (http://links.lww.com/ALN/B865). Each gene expression was normalized to the housekeeping gene acidic ribosomal phosphoprotein P0 (*36B4*) and expressed as fold change relative to nonventilated animals, using the 2–ΔΔCt method, where ΔCt = Ct (target gene) – Ct (reference gene).[30]

Statistical Analysis

Sample size calculation is provided in Supplemental Digital Content 1 (http://links.lww.com/ALN/B863). The primary outcome was the difference in gene expression of interleukin-6 among ventilator strategies, whereas the secondary outcomes were lung function, heterogeneity index, diffuse alveolar damage score, and expression of genes related to alveolar stretch, epithelial and endothelial cell injuries, and extracellular matrix damage.

Functional and echocardiographic parameters were compared between groups and over time using a mixed linear model based on a random intercept for each animal followed by Bonferroni's test. Molecular parameters and diffuse alveolar damage scores were assessed with the Kruskal–Wallis test with Dunn's *post hoc* test. The mixed linear models were constructed in IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., USA). All other tests were performed in GraphPad Prism version 6.00 (GraphPad Software, USA). Significance was established at $P < 0.05$.

Results

All animals survived to the end of the experiment (final time); thus, there were no missing data of any kind. Mean arterial pressure remained at or above 70 mmHg throughout the experiments. At the final time, no statistically significant differences among groups were observed in the volume of fluids required to keep mean arterial pressure at or above 70 mmHg (control, 13.1 ± 3.3 ml; no adaptation time, 13.6 ± 2.5 ml; shorter adaptation time: 10.3 ± 2.0 ml; longer adaptation time, 11.5 ± 2.7 ml). Oxygenation increased over time in the following groups: no adaptation, shorter adaptation time, and longer adaptation time. At the final time, oxygenation was higher in the no adaptation and shorter adaptation time groups than in control. In the no adaptation and shorter adaptation time groups, Pa_{CO_2} declined during the course of mechanical ventilation and was lower in the shorter adaptation and longer adaptation time groups compared with control (table 2 in Supplemental Digital Content 3, http://links.lww.com/ALN/B866). V_T increased as RR was adjusted, according to our experimental protocol, which targeted a constant minute ventilation (table 3 in Supplemental Digital Content 4, http://links.lww.com/ALN/B867). Respiratory system plateau pressure, ΔP_L, $Energy_L$ and $Power_L$ increased over time in the no adaptation, shorter adaptation time, and Longer adaptation time groups (table 1).

Cumulative power was greater in the longer adaptation time group than in the other groups at 2 h (fig. 1 in Supplemental Digital Content 5, http://links.lww.com/ALN/B864; fig. 2 in Supplemental Digital Content 6, http://links.lww.com/ALN/B868) and 1 h (fig. 3 in Supplemental Digital Content 7, http://links.lww.com/ALN/B869).

At 2 h, the diffuse alveolar damage score, which represents the severity of atelectasis, ductal overdistension, interstitial edema, inflammation, and airway detachment, was increased in the longer adaptation time group compared with the control and shorter adaptation time groups due to statistically significant increases in atelectasis, interstitial edema, and detachment of airway epithelium (table 2; fig. 4 in Supplemental Digital Content 8, http://links.lww.com/ALN/B870). At 1 h, diffuse alveolar damage was higher in the longer adaptation time group than in the control and shorter adaptation time groups (fig. 5 in Supplemental Digital Content 9, http://links.lww.com/ALN/B871; table 4 in Supplemental Digital Content 10, http://links.lww.com/ALN/B872).

Heterogeneity index (β) was greater in the longer adaptation time group than in the control and shorter adaptation time groups at 2 h (fig. 2) and at 1 h (fig. 6 in Supplemental Digital Content 11, http://links.lww.com/ALN/B873). Gene expression of biomarkers associated with inflammation (interleukin-6), mechanical pulmonary stretch (amphiregulin), and extracellular matrix damage (metalloproteinase-9) in lung tissue were greater in the no adaptation and longer adaptation time groups compared with both the control and shorter adaptation time groups (fig. 3).

Gene expression of club cell secretory protein-16 (marker associated with epithelial cell damage) was greater in the longer adaptation group than in the shorter adaptation time group and decreased in the shorter adaptation time group as compared with the no adaptation time group. Gene expression of syndecan (marker associated with extracellular matrix connection to epithelial cells) was greater in the no adaptation time group than in control animals and reduced in longer adaptation and shorter adaptation time groups compared with the no adaptation time group. Decorin (marker associated with fibrosis) and vascular cell adhesion molecule 1 (marker associated with endothelial cell damage) expression did not differ among groups (fig. 7 in Supplemental Digital Content 12, http://links.lww.com/ALN/B874). At 1 h, interleukin-6 expression was increased in the longer adaptation time group compared with the control and shorter adaptation time groups. Club cell secretory protein-16, metalloproteinase-9, syndecan, decorin, and vascular cell adhesion molecule 1 expressions did not differ among groups (fig. 8 in Supplemental Digital Content 13, http://links.lww.com/ALN/B875).

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Table 1. Respiratory Parameters during Mechanical Ventilation

	Initial	30 min	60 min	90 min	Final	Time Effect	Group Effect
$Pplat_{,RS}$, cm H_2O						$P < 0.0001$	$P < 0.0001$
Control	10.6 ± 2.7	11.3 ± 2.2	11.5 ± 2.4	11.6 ± 2.1	12.5 ± 2.7		
No adaptation time	11.2 ± 2.2	10.6 ± 2.6	11.9 ± 2.1	28.2 ± 10.8*	28.2 ± 11.0*	†§‡	
Shorter adaptation time	11.8 ± 1.8	12.2 ± 1.4	29.4 ± 8.3*‖	28.1 ± 8.8*	26.8 ± 6.5*	†§	
Longer adaptation time	11.4 ± 1.5	18.2 ± 1.8*‖#	29.2 ± 8.5*‖	26.7 ± 7.6*	27.2 ± 9.5*	†	
$\Delta P_{,RS}$, cm H_2O						$P < 0.0001$	$P < 0.0001$
Control	6.8 ± 2.5	7.7 ± 2.0	7.9 ± 2.3	8.2 ± 1.7	8.9 ± 2.6		
No adaptation time	7.5 ± 1.9	7.2 ± 2.4	8.5 ± 2.0	25.2 ± 10.7*	25.3 ± 11.0*	†§‡	
Shorter adaptation time	8.2 ± 1.8	8.6 ± 1.4	26.1 ± 8.3*‖	24.8 ± 9.0*	23.5 ± 6.5*	†§	
Longer adaptation time	7.7 ± 1.6	14.9 ± 1.7*‖#	26.0 ± 8.5*‖	23.4 ± 7.8*	24.1 ± 9.3*	†	
$\Delta P_{,L}$, cm H_2O						$P < 0.0001$	$P < 0.0001$
Control	6.0 ± 2.6	6.8 ± 2.3	6.7 ± 2.4	7.3 ± 2.0	7.9 ± 2.7		
No adaptation time	6.7 ± 1.9	6.4 ± 2.3	7.4 ± 1.9	22.8 ± 10.6*	24.8 ± 10.5*	†§‡	
Shorter adaptation time	7.2 ± 1.7	7.5 ± 1.5*‖	23.4 ± 9.2*‖	22.7 ± 9.6*	21.0 ± 7.0*	†§	
Longer adaptation time	7.0 ± 1.5	13.4 ± 1.2*‖#	23.3 ± 7.9*‖	20.8 ± 7.3*	21.5 ± 8.7*	†	
$Energy_{,L}$, mJ						$P < 0.0001$	$P < 0.0001$
Control	0.6 ± 0.2	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.2	0.9 ± 0.3		
No adaptation time	0.8 ± 0.2	0.8 ± 0.3	0.9 ± 0.4	10.2 ± 6.2*	10.9 ± 5.7*	†§‡	
Shorter adaptation time	0.8 ± 0.2	0.8 ± 0.2	9.0 ± 4.0*‖	9.0 ± 4.4*	8.3 ± 3.3*	†§	
Longer adaptation time	0.8 ± 0.2	3.4 ± 0.7	9.9 ± 4.8*‖	8.9 ± 4.3*	9.5 ± 5.3*	†	
$Power_{,L}$, mJ/min						$P < 0.0001$	$P < 0.0001$
Control	47.3 ± 19.9	53.9 ± 18.7	52.5 ± 19.7	58.0 ± 18.2	61.9 ± 23.3		
No adaptation time	52.8 ± 14.8	50.3 ± 16.5	59.0 ± 16.9	179.5 ± 87.4*	188.6 ± 87.1*	†§‡	
Shorter adaptation time	57.1 ± 13.5	59.7 ± 12.8	186.1 ± 79.3*‖	182.8 ± 85.1*	170.8 ± 64.3*	†§	
Longer adaptation time	56.1 ± 12.3	105.7 ± 9.4*‖	183.8 ± 68.2*‖	164.3 ± 60.8*	173.4 ± 75.8*	†	

Respiratory parameters at initial time, 30 min, 60 min, 90 min, and final time in the following groups: mechanical ventilation with a protective strategy (V_T = 6 ml/kg) for 2 h (control); V_T = 6 ml/kg during hour 1 followed by an abrupt increase of V_T = 22 ml/kg until 2 h (no adaptation time); V_T = 6 ml/kg during the first 30 min followed by a gradual V_T increase up to 22 ml/kg for 30 min, then constant V_T = 22 ml/kg until 2 h (shorter adaptation time); and a more gradual V_T increase, from 6 to 22 ml/kg during 1 h followed by V_T = 22 ml/kg until 2 h (longer adaptation time). The values are means ± SD of eight animals in each group. Comparisons were done using a generalized linear model ($P < 0.05$). Time *versus* group effect: $P < 0.0001$.

**versus* control. ‖*versus* no adaptation time group. #*versus* shorter adaptation time group. †*versus* initial. §*versus* 30 min. ‡*versus* 60 min.

$Pplat_{,RS}$, respiratory system plateau pressure; $\Delta P_{,L}$, transpulmonary driving pressure; $\Delta P_{,RS}$, respiratory system driving pressure.

Table 2. Diffuse Alveolar Damage Score Variables

	Control	No Adaptation Time	Shorter Adaptation Time	Longer Adaptation Time
Atelectasis	9 (6–9)	4 (4–6)*	4 (2–6)*	12 (12–12)*‖#
Ductal overdistension	4 (4–6)	9 (8–12)*	6 (4–8)	4 (2–4)‖
Interstitial edema	4 (4–4)	4 (4–6)	2 (2–2)*‖	9 (8–9)*‖#
Inflammation	6 (6–9)	9 (6–9)	6 (6–9)	8 (8–12)
Airway detachment	4 (2–4)	6 (4–6)	2 (1–2)‖	8 (6–9)*#
DAD	28 (24–30)	34 (30–39)	20 (18–24)‖	43 (38–46)*#

Diffuse alveolar damage scores (DAD; scores arithmetically averaged from two independent investigators) representing injury from atelectasis, ductal overdistension, interstitial edema, inflammation, and airway detachment are shown. These features were measured in each of the following groups: mechanical ventilation with a protective strategy (V_T = 6 ml/kg) for 2 h (control); V_T = 6 ml/kg during hour 1, followed by an abrupt increase to V_T = 22 ml/kg throughout hour 2 (no adaptation time); V_T = 6 ml/kg during the first 30 min followed by a gradual V_T increase up to 22 ml/kg for 30 min, then constant V_T = 22 ml/kg during hour 2 (shorter adaptation time); and a more gradual V_T increase, from 6 to 22 ml/kg during 1 h, followed by V_T = 22 ml/kg during hour 2 (longer adaptation time). The values are given as medians and interquartile ranges of eight animals in each group. The comparisons among all groups were done by ANOVA on ranks with Dunn's *post hoc* test.

**versus* control ($P < 0.05$). ‖*versus* no adaptation time group ($P < 0.05$). #*versus* shorter adaptation time group ($P < 0.05$).

At the final time, echocardiography showed lower cardiac output in the no adaptation time group compared with control. Other cardiac parameters did not differ among groups nor over time (table 5 in Supplemental Digital Content 14, http://links.lww.com/ALN/B876).

Discussion

In the model of mild ARDS used herein, different strategies of V_T increase were compared to assess lung injury resulting from increased strain. Increasing strain gradually (shorter adaptation time) rather than abruptly (no adaptation time)

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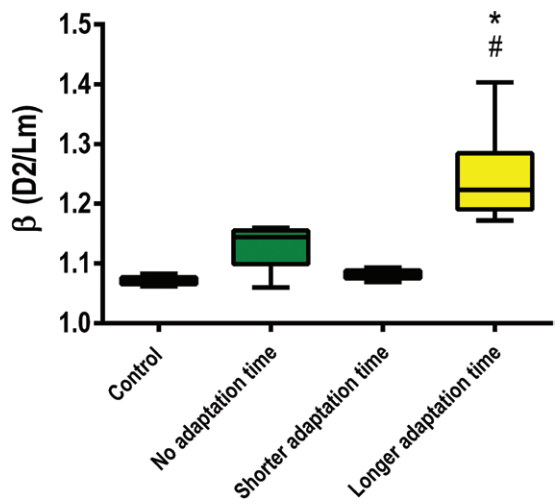

Fig. 2. Heterogeneity index (β) = D2/Lm in the following groups: mechanical ventilation with a protective strategy (V_T = 6 ml/kg) for 2 h (control); V_T = 6 ml/kg during hour 1 followed by an abrupt increase to V_T = 22 ml/kg throughout hour 2 (no adaptation time); V_T = 6 ml/kg during the first 30 min followed by a gradual V_T increase up to 22 ml/kg for 30 min, then constant V_T = 22 ml/kg during hour 2 (shorter adaptation time); and a more gradual V_T increase, from 6 to 22 ml/kg during hour 1, followed by V_T = 22 ml/kg during hour 2 (longer adaptation time). *Boxes* show the interquartile (25 to 75%) range, *whiskers* encompass the range (minimum to maximum), and *horizontal lines* represent median values of eight animals/group. **versus* control ($P < 0.05$); #*versus* shorter adaptation time group ($P < 0.05$). D2, central moments of mean linear intercept; Lm, mean linear intercept.

attenuated lung injury, likely by preemptive adaptation of the epithelial cells and extracellular matrix. However, a more gradual increase in V_T (longer adaptation time) compared with the shorter adaptation time led to more cumulative transfer of power and did not prevent lung damage, suggesting that the longer adaptation time strategy initiated injurious strain at an earlier time.

In experimental and clinical studies, mechanical ventilation with high V_T clearly promotes ventilator-induced lung injury.[13,17,31] In theory, excessive strain and ventilator-induced lung injury could be attenuated if V_T were increased slowly enough to reduce lung heterogeneity progressively and to allow the extracellular matrix to adapt to high V_T. This experimental study was designed to test that hypothesis by applying two potentially adaptive strategies in mild ARDS. In the ARDS model used herein, endotoxin was instilled intratracheally,[12,20,32,33] imposing a first hit with the intent of inducing or priming the lung for inflammation. ARDS animals allowed a longer adaptation time unexpectedly experienced the converse, showing increased heterogeneity index and interleukin-6 gene expression when compared with those given a shorter adaptation time.

The results of these experiments appear generally consistent with the unifying concepts of energy load, mechanical power, and repetitively excessive tidal stresses on the development of ventilator-induced lung injury. They also point to the potential for ventilator-induced lung injury to be attenuated or delayed (despite greater exposure to cumulative power) by a well sequenced, gradual approach to the maximum tidal stress and in so doing may offer insights into defining the root mechanical cause of ventilator-induced lung injury. Although it is uncontested that tidal stresses and strains that exceed a rather sharp threshold hold potential to cause lung injury,[34] our results indicate that some degree of stress accommodation might occur if the application of high driving and plateau pressures were made gradually rather than abruptly. The exact mechanism of ventilator-induced lung injury at the cellular and extracellular matrix levels at different adaptation times remains to be precisely defined.[11]

Both the absence of adaptation time and a longer run-up to high V_T increased expression of interleukin-6 and amphiregulin, which are early surrogates of inflammation[35] and alveolar stretch,[26] respectively. Amphiregulin expression is positively modulated by lung overdistension, and activates chemokines, cytokines, and adhesion molecules.[12,26,34] In this study, amphiregulin expression was accompanied by ductal overdistension in animals with stepwise presentation of high V_T and, unexpectedly, by airway detachment in the Longer adaptation time group. Club cell secretory protein-16, a protein mainly produced and secreted by club cells in the respiratory or terminal bronchioles, has been proposed as a biomarker of lung epithelial injury,[36] and its expression was also increased in the longer adaptation time group. Interestingly, vascular cell adhesion molecule 1, a marker of endothelial cell injury, did not vary among groups. Even though high V_T resulted in endothelial cell damage in healthy lungs,[37] in the mild ARDS model used herein, the increase in V_T led predominantly to greater epithelial cell injury.

The damage caused using a longer adaptation time to reach high V_T was not limited to epithelial cells; it also affected extracellular matrix components. Repeated high stress and strain above a given threshold crossed before V_T of more than 22 ml/kg may displace or rupture the extracellular matrix.[31,38,39] In the presence of an abrupt increase to high V_T or of a protracted run-up time, metalloproteinase-9 expression has been shown to increase statistically significantly. Metalloproteinase-9 cleaves collagen and increases neutrophil influx, thus contributing to lung inflammation.[40] Expression of syndecan-1, a cell-surface heparan sulfate proteoglycan primarily elaborated by the lung epithelium and involved in regulation of lung repair, remodeling,[27] and inflammation,[41] was higher only when V_T increased abruptly. V_T variations predicted changes in syndecan-1 expression,[13] highlighting the sensitivity of this biomarker to detect challenges to extracellular matrix homeostasis. Decorin, a marker of cell proliferation

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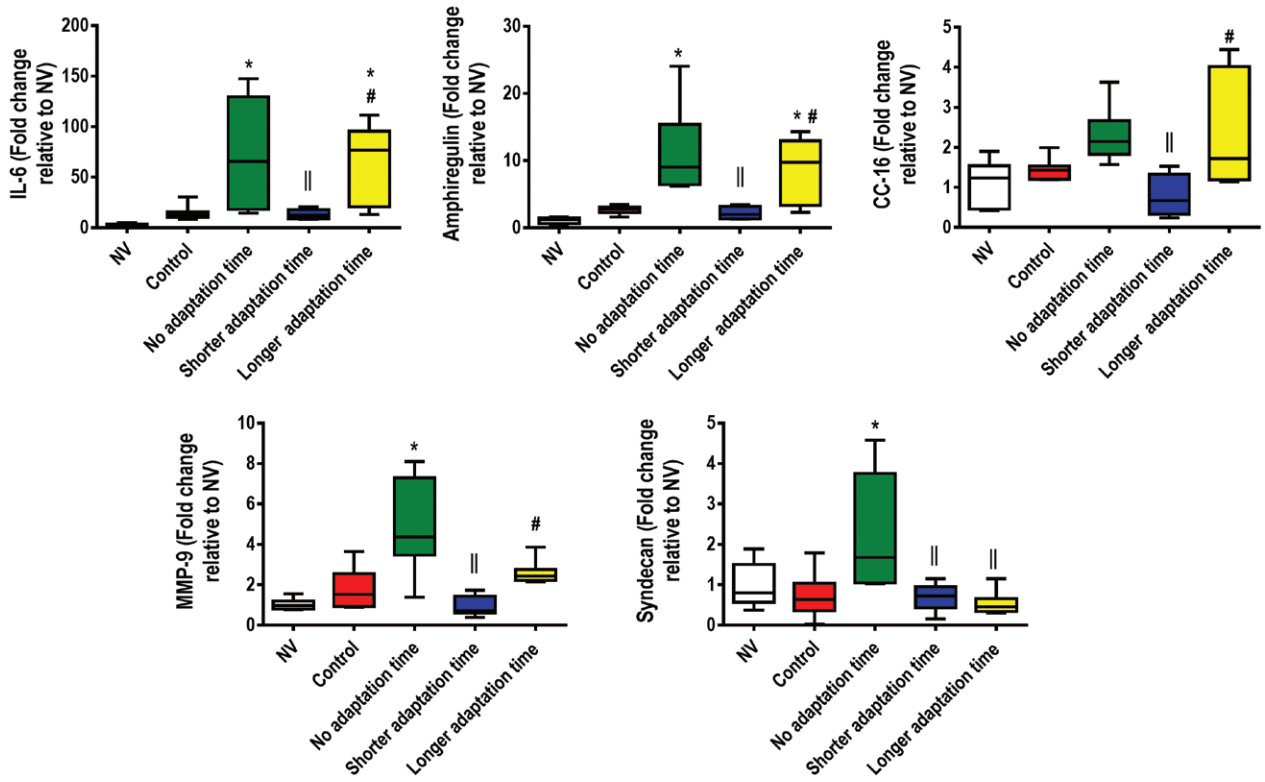

Fig. 3. Expression of biologic markers associated with inflammation (interleukin [IL]-6), alveolar pulmonary stretch (amphiregulin), epithelial cell damage (club cell protein 16 [CC-16]), metalloproteinase (MMP)-9, and extracellular matrix connection to epithelial cells (syndecan-1) in the following groups: mechanical ventilation with a protective strategy (V_T = 6 ml/kg) for 2 h (control); V_T = 6 ml/kg during hour 1 followed by an abrupt increase to V_T = 22 ml/kg during hour 2 (no adaptation time); V_T = 6 ml/kg during the first 30 min followed by a gradual V_T increase up to 22 ml/kg for 30 min, then constant V_T = 22 ml/kg during hour 2 (shorter adaptation time); and a more gradual V_T increase, from 6 to 22 ml/kg during hour 1 followed by V_T = 22 ml/kg during hour 2 (longer adaptation time). Relative gene expression was calculated as a ratio of the average gene expression levels compared with the reference gene (acidic ribosomal phosphoprotein P0 [*36B4*]) and expressed as fold change relative to nonventilated animals (NV). *Boxes* show the interquartile (25 to 75%) range, *whiskers* encompass the range (minimum to maximum), and *horizontal lines* represent median values of eight animals/group. **versus* control ($P < 0.05$); ‖*versus* no adaptation time group ($P < 0.05$); #*versus* shorter adaptation time group ($P < 0.05$).

inhibition and collagen synthesis, did not change to a statistically significant extent among groups. Our findings suggest that abrupt increases in V_T may cause extracellular matrix fragmentation,[31,38] thus resulting in lung inflammation. To help explain the unanticipated disparity between adaptation strategies, we speculate that shorter adaptation time reduced heterogeneity index, overdistension, and matrix degradation without additional exposure to repeated injurious stresses and energy exposures that occurred in the more slowly ramped-up V_T of the longer adaptation time group.

The viscoelastic effects of stress redistribution, stress relaxation, and "creep" can be observed in the difference between pressure at zero flow and the steady-state plateau pressure when an end-inspiratory pause is sustained.[42] Although this process is quickly evident in injured lungs within the span of a single tidal cycle, complete accommodation requires longer to develop.[43]

Our prior work showing that a slow approach to a high-pressure recruitment maneuver is associated with less injury[44] indicates a protracted time scale of adaptation in response to escalating PEEP. In the present study, however, PEEP was held constant, because the focus was on the accommodation to escalating V_T.

The integrative power exposure theory of ventilator-induced lung injury appears consistent with our findings[12,45]; however, raw power can be applied in various ways. The Shorter adaptation time group encountered greater cumulative power exposure but developed less injury compared with the group with no adaptation, which suggests that the same level of mechanical power may be safely applied if delivered with tidal strains held below a certain level. Moreover, the span over which the rising energy load was building to reach its peak very likely allowed simultaneous stress relaxation to help disperse strain, improving tolerance. When comparing

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the shorter adaptation time (less damage) and longer adaptation time (greater damage) groups, increased power exposure in the latter is a possible contributor but of itself seems unlikely to be the sole agent of injury. Despite marginal differences in cumulative power, there was a major difference in the degree of injury between these two adaptation groups. Recognizing that a sharp or indistinct tidal strain level must be crossed before injury develops, one possibility to explain these observations is that repetitive above-threshold straining cycles were the main stimulus for injury and that the group with longer adaptation time sustained more of these over the time course of the experiments. It remains unclear whether it is the energy that is delivered above strain-injury threshold or repetitive excessive tidal strain cycling itself that progressively breaks down the extracellular matrix, damages epithelial cells, and degrades the structural microelements of the basement membrane in stepwise fashion. What is apparent after comparing shorter and longer adaptation time groups is that any initial accommodation to stress waned with duration of injurious stress.

One attractive way to piece these observations into a coherent mechanism is to understand that injury and adaptation proceeded along separate timelines: favorable influences of stress relaxation and redistribution of air within the lungs were eventually overwhelmed by the accumulating number of damaging stress cycles in excess of the damaging threshold. An additional mechanism, alveolar–capillary barrier repair, may also have aided stress accommodation in the early phase of increasing V_T. Repair of transient alveolar capillary permeability in rodents may occur in less than 5 min.[17] Moreover, lipid trafficking to and from the cellular plasma membrane, the incorporation of intracellular lipid vesicles in the existing plasma membrane,[46] and changes in cytoskeletal structure may also explain the differences between groups with shorter and longer adaptation times.[47]

Limitations

This study has noteworthy limitations. As a set of acute experiments conducted in a small-animal model of mild ARDS, these results clearly cannot be applied immediately to patient care. These findings apply to the specific experimental circumstances and time intervals used herein. Furthermore, the duration of experiments was not sufficient to determine whether injury was attenuated or simply delayed by the shorter adaptation period. We cannot rule out that other variables or combinations of variables might result in other outcomes. Future studies are required to confirm whether a hard threshold of transition to injury exists, to determine the level of any such threshold in this model of mild ARDS, and to evaluate strategies that slowly increase PEEP or respiratory rate. Nonetheless, apart from any mechanistic insights, these experimental data do have potential clinical relevance; small V_T values and restrained driving pressures are sometimes impossible to apply without initiating other aggressive measures to assure adequate pH and gas exchange. Our results should be considered evidence in support of a novel principle of lung protection—gradual application of a proposed increase in V_T and stress with awareness of levels that approach the threshold of injury.

Conclusions

In experimental mild ARDS, lung damage in the shorter adaptation time compared with the no adaptation time group was attenuated by preemptive adaptation of the alveolar epithelial cells and extracellular matrix. A longer adaptation time compared with the shorter adaptation time was associated with increased cumulative power and did not prevent lung damage, perhaps because an injurious strain threshold was reached earlier, and such strain was imparted for longer; this may have overcome the adaptation afforded by a briefer exposure to high-strain tidal cycles and outweighed any benefit. The present data support the concept that ventilator-induced lung injury relates closely to repetitive cycling above a strain-injury level.

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Competing Interests

The authors declare no competing interests.

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A year after co-discovering chloroform, Germany's Justus Liebig synthesized its chemical cousin, the sedative chloral (hydrate), in 1832. Another German, apothecary Maximilian "Max" L. Frankenstein (1849 to 1898), immigrated to Fort Wayne, Indiana, to practice as a pharmacist (one of his Fort Wayne bottles, *above*). After then moving to Chicago to study at Rush Medical College, Frankenstein began sedating himself nightly, as a senior medical student, with chloral to combat insomnia. Discovered unconscious the morning of October 1, 1898, Frankenstein died less than 16 h later from his accidental chloral overdose. Could he have been saved, had he read *Dracula*? That novel was published in London before Max died, but not in America until after his passing. Indeed, while writing *Dracula*, author Bram Stoker had created one character who cautioned chloral users that they "should be careful not to let it grow into a habit." (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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